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# Shorter Notes

**Chemotaxonomic survey of flavonoids from *Sphaerostephanos* (Thelypteridaceae) of Peninsular Malaysia.**—*Sphaerostephanos* (Thelypteridaceae) is characterized by reduced basal pinnae, sessile non-resinous spherical yellow glands on the upper leaf surface, and a base chromosome number of x=36 (Holttum, R. E., *Flora Malesian. Series II. Pteridophyta. Ferns and Fern Allies. Vol I. pt. 5, Thelypteridaceae*. M. Nijhoff, The Hague. 1982). Little is known about the flavonoid chemistry of Peninsular Malaysia *Sphaerostephanos* species. In this study of the flavonoid distribution of pinnae, we contribute valuable information on inter-specific relationships. Leaves from freshly dried plant material collected from various habitats in Peninsular Malaysia were analysed. Voucher specimens of the ferns (collector number: *UKY 232*, *UKY 288*, *UKY 289*, *UKY 293*, *UKY 296*, *UKY 297*, *UKY 319*, *UKY 312*, *UKY 323*, *UKY 326*, *UKY 333*, *UKY 335*, *NMJ 1*, *NMJ 2*, *NMJ 7*, *NMJ 10*, *NMJ 11*, *NMJ 13*, *NMJ 15*, *NMJ 19*) have been deposited in the herbarium of the Department of Biology of the Universiti Putra Malaysia, Serdang, Selangor. Standard chromatographic procedures (Harborne, J. B. 1967, *Comparative Biochemistry of the Flavonoids*, Academic Press, London; Harborne, J. B. 1984, *Phytochemical methods*, Chapman and Hall, London; Markham, K. R. 1982, *Techniques of flavonoid Identification*, Academic Press, London) were used for examining flavonoids present in direct and acid hydrolysed leaf extracts; the common aglycones were identified by means of $R_f$ values and color reaction in UV light when compared with standard markers. In acid-hydrolyzed extracts, the flavones were recognized by their distinct, dark yellow spots on paper chromatograms in UV light. When fumed with ammonia vapor they became bright yellow. The flavonols appeared yellow in UV light before and after fuming with ammonia. For complete identification of flavonoid glycosides, samples were separated in one-dimensional chromatograms of direct extracts and then the pure flavonoids were identified by UV spectral analysis using standard procedures of Mabry and coworkers (*The Systematic Identification of the Flavonoids*, Springer-Verlag, New York, 1970). In addition to spectral techniques, flavonoids were identified by PC (Whatman No. 1) co-chromatography of the glycosides and products of enzyme and acid hydrolyses in *n*-butanol-acetic acid-water (BAW, 4:1:5) and 50% glacial acetic acid (50%HOAc). The aglycones were identified by TLC (Merck) co-chromatography in BAW, forestal (concentrated hydrochloric acid-acetic acid-water, 3:30:10) and 30%HOAc, whereas the sugars were identified by PC co-chromatography in BAW, *n*-butanol-ethanol-water (BEW, 4:1:2.2) and toluene-η-butanol-pyridine-water (TBPW, 5:1:3:3).

Twenty populations representing nine species were examined for intraspecific flavonoid variation, but no significant qualitative population level variation was observed in the species studied. Six of the nine *Sphaeroste-*

*phanos* species examined contain quercetin (Qu) and two of these six also possess kaempferol (Km). Samples of *S. pterocarpus*, *S. norisii* and *S. peltochlamys* do not contain flavonols, but apigenin was found in *S. hendersonii* and *S. heterocarpus*. Isorhamnetin was detected in *S. penniger*, and as far as the authors are aware this is the first report of the presence of isorhamnetin in the Thelypteridaceae.

Ten flavonoids were isolated and purified in this investigation. These compounds were glycosides of flavones and flavonols (Table 1). Flavonol *O*-glycosides appear to be common components of *Sphaerostephanos* (found in 66.6% of the species studied). Thus, quercetin 3-*O*-glucoside, quercetin 3-*O*-galactoside, kaempferol 3-*O*-glucoside, kaempferol 3-*O*-rhamnoside and kaempferol 3-*O*-galactoside were respectively found in 22%, 33%, 22%, 44% and 22% of the species studied. Isorhamnetin 3-*O*-glucoside was detected only in *S. heterocarpus*. Flavone *C*-glycosides and flavone *O*-glycoside seem to have a restricted distribution in this genus and were found in only 22% and 11% of the *Sphaerostephanos* species investigated. Schaftoside and isoschaftoside were found in *S. polycarpus* and apigenin 7-*O*-glucoside was detected in *S. heterocarpus*. In addition, the presence of isorhamnetin 3-*O*-glucoside in *S. hendersonii* and apigenin 7-*O*-glucoside in *S. heterocarpus* is also now reported for the first time in the Thelypteridaceae.

Despite the small sample size, species of *Sphaerostephanos* appear to be divisible into two groups based on the presence of flavonoid glycosides and aglycones (Table 2). However, a more comprehensive survey of this genus is required in order to establish this difference conclusively. Species of Group A contain both flavonoid glycosides and aglycones, whereas species of Group B, completely lack flavonoids. The species of Group A do vary in their glycosides such that it is possible to distinguish some of them by their flavonoid patterns. Two species of *Sphaerostephanos*, *S. polycarpus* and *S. unitus* contain kaempferol, quercetin and kaempferol 3-*O*-rhamnoside. *Sphaerostephanos heterocarpus* accumulates apigenin 7-*O*-glucoside, kaempferol 3-*O*-glucoside and isorhamnetin 3-*O*-glucoside.

Although *S. polycarpus* and *S. unitus* both produce quercetin 3-*O*-glucoside, they can be chemically distinguished from one another because *S. polycarpus* accumulates schaftoside and isoschaftoside whereas *S. unitus* accumulates quercetin 3-*O*-galactoside and kaempferol 3-*O*-galactoside. The last two species, *S. penniger* and *S. larutensis*, have quercetin and quercetin 3-*O*-galactoside, but *S. penniger* produces isorhamnetin as well.

In conclusion, the present work establishes that glycosides of kaempferol are the major flavonoids of *Sphaerostephanos*, being present in 44% of the species examined. Moreover, *Sphaerostephanos* can be subdivided into two groups based on the presence of flavonoids in frond extracts. How far this or any other chemical data can be used to assess the validity of the many competing taxonomic arrangements of the species within *Sphaerostephanos* can only be determined after many more species from other geographical areas have been examined.

TABLE 1. Identification of flavonoid glycosides from the pinnae of *Sphaerostephanos* sp.

| Flavonoid glycosides | Color in UV/UV + $NH_3$ | BAW | $H_2O$ | 15% HOAc | PhOH | UV spectrum/nm in | | | |
|---|---|---|---|---|---|---|---|---|---|
| Quercitin 3-glucoside | dark/yellow | 67 | 27 | 54 | 49 | 425. 357. 256 | 267 | 379 | 404 |
| Schaftoside | dark/yellow | 72 | 30 | 58 | 52 | 353, 288 | – | – | – |
| Isoschaftoside | dark/yellow | 72 | 29 | 59 | 87 | 350, 292 | – | – | – |
| Km 3-rhamnoside | dark/yellow | 86 | 28 | 51 | 72 | 352, 267 | 273 | 355 | 392 |
| Quercetin 3-glucoside | dark/yellow | 72 | 15 | 39 | 59 | 359, 267 | 272 | 379 | 388 |
| Kaempferol 3-glucoside | dark/yellow | 79 | 19 | 41 | 78 | 350, 295sh, 266 | 269 | 350 | 390 |
| Kaempferol 3-galactoside | dark/yellow | 77 | 13 | 43 | 73 | 350, 267 | 267 | 350 | 385 |
| Kaempferol 3-rutinoside | dark/yellow | 65 | 31 | 61 | 67 | 350, 268 | 270 | 360 | 402 |
| Apigenin 7-glucoside | dark/yellow | 68 | 10 | 30 | 76 | 336, 265 | 265 | 345 | 381 |
| Isorhamnetin 3-glucoside | dark/yellow | 67 | 24 | 43 | 49 | 350, 266 | 273 | 350 | 385 |

Key: sh. = shoulder; BAW = η-butanol:acetic acid:water (4:1:5).

TABLE 2. The distribution of flavonoids in the pinnae of *Sphaerostephanos* sp.

| Taxon and collector | Flavone *C*-glycosides | | | Flavones and Flavonols | | | | Flavonol *O*-glycosides | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | SCH | ISCH | Ap 7-glu | Km | Qu | AP | Isorham | Qu 3-glu | Qu 3-gal | Isorham 3-glu | Km 3-glu | Km3-gal | Km 3-gal |
| **Group A:** | | | | | | | | | | | | | |
| *S. heterocarpus* (Bl.) Holtt. | − | − | + | − | + | + | − | − | − | + | + | + | + |
| *S. hendersonii* Holtt. | − | − | + | − | + | + | − | − | − | + | + | + | |
| *S. polycarpus* (Bl.) Copel. | + | + | − | + | + | − | − | + | − | − | − | + | |
| *S. unitus* (L.) Holtt. | − | − | − | + | + | − | − | + | + | − | − | + | + |
| *S. penniger* (Hk.) Holtt. | − | − | − | − | + | − | + | − | + | − | − | − | − |
| *S. larutensis* (Bedd.) C. Chr. | − | − | − | − | + | − | − | − | + | − | − | − | − |
| **Group B**: | | | | | | | | | | | | | |
| *S. norisii* (Rosenst.) Holtt. | − | − | − | − | − | − | − | − | − | − | − | − | − |
| *S. peltochlamys* (C. Chr.) Holtt. | − | − | − | − | − | − | − | − | − | − | − | − | − |
| *S. pterocarpus* (v.A.v.R.) Holtt. | − | − | − | − | − | − | − | − | − | − | − | − | − |

Key: SCH = schaftoside; ISCH = isoschaftoside; Ap 7-glu = apigenin 7-glucoside; Km = kaempferol; Qu = quercetin; Isorham = isorhamnoside; Qu 3-glu = quercetin 3-glucoside; Qu 3-gal = quercetin 3-galactoside; Isorham 3-glu = isorhamnetin 3-glucoside; Km 3-glu = kaempferol 3-glucoside; Km 3-rha = kaempferol 3-rhamnoside; Km 3-gal = kamepferol 3-galactoside.

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